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13. ABSTRACT ***(Maximum 200 Words)***

The goal of our project is to find novel treatments for breast cancer metastasis. We view metastasis as a breakdown of mechanisms that govern tissue organization. We postulated that metastasis is initiated by molecular cues that improperly stimulate cancer cell motility. Therefore, our approach is to block metastasis by understanding, and then interfering with, molecular and cellular mechanisms that regulate cell motility. We found that, in the mammary gland, several of these mechanisms revolve around the interaction of matrix metalloproteases (MMP) with laminin-5 (Ln-5), an extracellular matrix protein of the breast gland basement membrane. One major finding in this past year was the identification of the Ln-5 site onto which cells adhere and migrate. We also defined the position, relative to this site, of the docking site for antibodies that block cell motility. A second important finding is the location of Ln-5 sites that are cleaved by MMPs, and the compositon of the Ln-5 fragments resulting from this proteolytic activity. This information is critical to design in vivo animal experiments in which we will test the ability of antibodies to Ln-5, or Ln-5 fragments, to block tumor cell motility and hopefully metastasis.

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FOREWORD

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PI - Signature 7/17/00 Date

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Progress Report 1999-2000 ***(REVISED)***
Idea Grant DAMD 17-98-1-8150
P.I. Vito Quaranta

Introduction (paraphrased from last year's):

Our long-term goal is to find new treatments for breast cancer metastasis, based on targeting cellular and molecular mechanisms underlying the process of metastasis itself. The element of innovation in our approach is that we visualize metastasis as a problem of breakdown in tissue organization. Consequently, for the purposes of cancer treatment, our target is the mammary gland as a tissue, rather than the individual cells. By taking a reductionist approach, we investigate the actual molecular mechanisms that keep breast epithelial cells segregated on the luminal side of the basal lamina. These cells are the ones from which invasive breast cancer arises. We previously identified a molecular mechanism (1, 2) that determines whether normal or cancer breast cells may cross the basal lamina. This mechanism relies on the interaction of laminin-5, a major extracellular matrix molecule of basal lamina, with matrix metalloproteases and integrins. The challenge of this proposal is to determine how dominant this mechanism is in regulating migratory versus stationary behavior of breast epithelial cells. Should we find that such mechanism is rate limiting for metastasis, we will be close to a discovery phase for novel drugs or treatments that may prevent or block breast cancer invasion.

Body:

AIM 1. To inhibit mammary epithelial cell motility in vitro and cell metastasis in vivo by blocking the migratory site of laminin-5 (Ln-5).

MIG-1 is an antibody that blocks migration of cancer cells on Ln-5 cleaved by metalloproteases (MMPs). We proposed to map the binding site of this antibody on the Ln-5 molecule, since it might unveil structural features of Ln-5 that stimulate migration. We have now completed the mapping of the MIG-1 epitope on the LG2 domain of the Ln-5 α3 subunit. By site-directed mutagenesis, we showed that the epitope is restricted to a stretch of 6 amino acids towards the center of the molecule. Recently, the three-dimensional structure of the Ln-2 α2LG5 domain was solved. This domain is structurally homologous to α3LG2. Therefore, we were able to model the primary sequence of LG2 over the structure of LG5. This comparison predicted that the MIG-1 epitope would be located in a loop of the LG5 domain which is exposed to the aqueous environment, corroborating our mapping results.

To prove that the LG2 domain is involved in cell adhesion and migration, we also mapped the integrin binding site of Ln-5. Unexpectedly, the integrin binding site maps not to LG2, but rather to the LG3 domain, which is spatially close to LG2. This result suggest a mechanism whereby the LG2 and LG3 domain interact with each other and with integrins in order to support adhesion and migration. This mechanism is currently being investigated.

AIM 2. To inhibit mammary epithelial cell motility in vitro and cell metastasis in vivo by inhibiting the cleavage of Ln-5 by MMP2.

During last year, we found that MMP2 is not the only protease to cleave Ln-5. MT1-MMP, a surface bound MMP, cleaves Ln-5 at the same site as MMP2, as well as to another site, approximately 150 amino acids upstream (1). The interesting fact is that the combined actions of MMP2 and MT1-MMP liberate a fragment of the Ln-5 γ2 chain, whose structure resembles that of EGF (epidermal growth factor). EGF and EGF-like ligands are well known to display mitogenic and motogenic activity. We have made recombinant proteins spanning the cleavage sites of these MMPs, as well as the liberated fragment. By using these recombinant fragments with appropriate tags, we have confirmed that both MMP2 and MT1-MMP cleave them in vitro. Furthermore, some of these fragments interfered with cell migration in in vitro assays. An intriguing result is that the liberated fragment binds to the cell surface and stimulated tyrosine phosphorylation, suggesting it may bind to a receptor. These results strongly encourage testing these recombinant fragments in vivo, which we plan to carry out this coming year.

Aim 3. To produce monoclonal antibodies that react with MMP2-cleaved Ln-5 and not with intact Ln-5, and to use them in immunohistological assays for correlating the location of cleaved Ln-5 with breast cancer cell invasion sites.

Using purified Ln-5 for this purpose has proven not feasible, because both intact and cleaved Ln-5 are present in purified preparations and are difficult to separate. We plan to change approach and use instead the recombinant fragments mentioned in the Aim above.

Key Research Accomplishments:

- Mapped integrin binding site on Laminin-5 to domain LG3, first time this was done on any laminins
- Mapped epitope for antibody MIG-1, which blocks cell migration, to Laminin-5 domain LG2
- Discovered that MT1-MMP is another proteolytic enzyme, besides MMP2, which cleaves Laminin-5
- Defined the boundaries of a Laminin-5 fragment that is proteolytically cleaved out by the action of MMPs
- Produced recombinant Laminin-5 MMP fragments

Reportable Outcomes:

- Manuscripts, abstracts, presentations
  Two manuscripts in print, see References below
  Presentation: 2000 Gordon Conference "Basement Membranes"
- Patents and licenses
  None
- Degrees obtained
  None
- Development of reagents
  Recombinant Domain III from Laminin-5
  Recombinant LG2 and LG3 domains from Laminin-5

- Informatics
  None
- Funding applied for
  NIH grant application RO1-GM46902 "Molecular Regulation of Integrin Functions by Laminin Domains", PI Vito Quaranta, based on results from Aims 1 and 2 of this grant.
- Employment
  None

Conclusions:

We mapped the Ln-5 binding site for antibodies that block adhesion and/or migration. In addition, we identified the domain of Ln-5 that binds integrins and supports cell adhesion and migration. Surprisingly, this domain is close to, but does not overlap with the binding site of the blocking antibodies. Nonetheless, this is an important insight towards our goal of designing reagents that inhibit the migratory site of Ln-5 and therefore may interfere with metastasis.

We have overcome limitations imposed by protein purification methods, and plan to produce cleaved Ln-5 monoclonal antibodies by using recombinant fragments as immunogens.

We have further defined Ln-5 fragments that are the targets of, or result from MMP cleavage, and are closer to test them in vivo for possible inhibitory effects on metastasis. This has been a long-standing question in the Ln-5 field, and therefore we expect our results to have significant impact. In spite of the fact that Ln-5 is clearly involved in metastasis, absence of structural details on its adhesion/migration domains has frustrated efforts to interfere with cancer invasion. By comparison, in the case of fibrinogen, another extracellular matrix molecule involved in blood clotting, knowledge of its adhesion site for platelets has led to the development of clotting pharmaceuticals that are already available to the public.

Similar considerations are applicable to our studies on the MMP2 cleavage site. MMP inhibitors are widely considered strong candidates as anti-metastasis drugs (2). In Aim 2, we have shown that a fragment of Ln-5 is cleaved by MMPs. This result was not obviously predictable, and puts us in a position to eventually use the Ln-5 fragment as a basis for the design of MMP inhibitors.

References:

1. Koshikawa, N., Giannelli, G., Cirulli, V., Miyazaki, K., and Quaranta, V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J. Cell Biol. *148:*615-624, 2000.

2. Quaranta, V. Cell migration through extracellular matrix: membrane-type metalloproteinases make the way. J. Cell Biol. *149:*1167-1170, 2000.

Appendices:

Letter regarding unpublished data.
Figure 1.
Figure 2.